

What is claimed is:

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- Rob C17
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1. An expression cassette comprising,  
a polynucleotide encoding *luxA*, *luxB*, *luxC*, *luxD* and *luxE* gene products,  
wherein (a) transcription of the polynucleotide results in a polycistronic RNA encoding  
all the gene products; (b) each of the *luxA*, *luxB*, *luxC*, *luxD* and *luxE* gene products is  
expressed as an individual polypeptide; and (c) polynucleotide sequences comprising  
Gram-positive ribosome-binding site sequences are located 5' to all of said *lux* coding  
sequences.
  2. The expression cassette of claim 1, further comprising a multiple-insertion site  
located 5' to said *luxA*, *luxB*, *luxC*, *luxD* and *luxE* coding sequences.
  3. The expression cassette of claim 1, wherein at least one Gram-positive  
ribosome binding site comprises the sequence presented as SEQ ID NO:1.
  4. The expression cassette of claim 1, wherein the coding sequences of the gene  
products are derived from *Photobacterium luminescens*.
  5. The expression cassette of claim 1, wherein the polynucleotide further  
comprises a promoter located 5' to all of said *lux* coding sequences wherein transcription  
of the polynucleotide results in a polycistronic RNA encoding all the *lux* gene products.
  6. The expression cassette of claim 5, wherein said promoter is contained in an  
Expression Enhancing Sequence selected from the group consisting of Sa1, Sa2, Sa3,  
Sa4, Sa5, and Sa6.
  7. The expression cassette of claim 5, wherein said promoter is contained in an  
Expression Enhancing Sequence selected from the group consisting of Sp1, Sp5, Sp6,  
Sp9, Sp16 and Sp17.

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8. The expression cassette of claim 7, wherein said promoter is contained in Expression Enhancing Sequence Sp16.

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21. An expression cassette comprising,

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10 a polynucleotide encoding *luxA*, *luxB*, and *luc* gene products, wherein (a) transcription of the polynucleotide results in a polycistronic RNA encoding all three gene products, (b) polynucleotide sequences comprising Gram-positive ribosome-binding site sequences are located adjacent the 5' end of the *luxA* coding sequences, adjacent the 5' end of the *luxB* coding sequences, and adjacent the 5' end of the *luc* coding sequences, and (c) each of the *luxA*, *luxB*, and *luc* gene products is expressed as an individual polypeptide.

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22. The expression cassette of claim 21, wherein said polynucleotide further encodes *luxC*, *luxD* and *luxE* gene products, wherein (i) Gram-positive ribosome-binding site sequences are located 5' to each of the *luxC*, *luxD*, and *luxE* coding sequences, and (ii) each of the *luxC*, *luxD*, and *luxE* gene products is expressed as an individual polypeptide.

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24. The expression cassette of claim 21, wherein the polynucleotide further comprises a promoter located 5' to all of said *lux* and *luc* coding sequences wherein transcription of the polynucleotide results in a polycistronic RNA encoding all the *lux* and *luc* gene products.

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25. The expression cassette of claim 24, wherein said promoter is contained in an Expression Enhancing Sequence selected from the group consisting of Sa1, Sa2, Sa3, Sa4, Sa5, and Sa6.

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26. The expression cassette of claim 24, wherein said promoter is contained in an Expression Enhancing Sequence selected from the group consisting of Sp1, Sp5, Sp6, Sp9, Sp16 and Sp17.

5 27. The expression cassette of claim 26, wherein said promoter is contained in Expression Enhancing Sequence Sp16.

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28. The expression cassette of claim 21, further comprising a multiple-insertion site located 5' to said *luxA*, *luxB*, *luc*, *luxC*, *luxD* and *luxE* coding sequences.

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29. The expression cassette of claim 21, wherein the coding sequences for *luxA* and *luxB* are obtained from *Photobacterium luminescens*.

15 34. The expression cassette of claim 1, wherein the expression cassette is contained within a bacterial transposon.

35. The expression cassette of claim 1, wherein the expression cassette is contained within a bacterial mini-transposon.

AS 20 36. The expression cassette of claim 1, wherein the coding sequences of the gene products comprise codons that are optimal for expression of the gene products in a host system into which the expression cassette is to be introduced.

AB 25 49. A shuttle vector comprising:  
an expression cassette according to claim 1;  
a polynucleotide encoding a selectable marker;  
a Gram-positive origin of replication; and  
a Gram-negative origin of replication.

56. A method of modifying a Gram-positive organism to produce light, comprising transforming the Gram-positive organism with an expression cassette according to claim 1.

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5 58. A method of screening an analyte for its ability to affect expression of a reporter marker, comprising:  
providing the analyte to Gram-positive bacteria comprising the luciferase expression cassette of claim 1, wherein said reporter marker comprises luciferase; and  
monitoring the effect of the analyte on the ability of the Gram-positive bacteria to  
10 produce light, thereby identifying whether the analyte affects expression of the reporter in Gram-positive bacteria.

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15 60. A method of screening an analyte for its ability to affect expression of a reporter marker in a living, non-human animal, comprising:  
introducing Gram-positive bacteria comprising the luciferase expression cassette of claim 1 into the animal, wherein said reporter marker comprises luciferase;  
providing the analyte to the animal; and  
monitoring the effect of the analyte on the ability of the Gram-positive bacteria to  
produce light, thereby identifying whether the analyte affects expression of the reporter in  
20 Gram-positive bacteria in the living, non-human animal.

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25 62. A Gram-positive bacteria capable of producing light, wherein (a) the bacteria comprises *luxA*, *luxB*, *luxC*, *luxD*, and *luxE* coding sequences, and (b) about  $1 \times 10^6$  bacterial cells can produce at least about  $1 \times 10^4$  Relative Light Units at about 37°C.

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64. A Gram-positive bacteria comprising an expression cassette according to claim 1.

68. The expression cassette of claim 1, wherein the arrangement of the coding sequences for the *lux* gene products is in the following relative order 5' - *luxA-luxB-luxC-luxD-luxE*- 3'.

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69. The expression cassette of claim 21, wherein the arrangement of the coding sequences for the *lux* gene products is in the following relative order 5' - *luxA-luxB-luxC-luxD-luxE*- 3'.

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70. The expression cassette of claim 21, wherein the expression cassette is contained within a bacterial transposon.

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71. The expression cassette of claim 21, wherein the expression cassette is contained within a bacterial mini-transposon.

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72. The expression cassette of claim 21, wherein the coding sequences of the gene products comprise codons that are optimal for expression of the gene products in a host system into which the expression cassette is to be introduced.

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73. A shuttle vector comprising:  
an expression cassette according to claim 21;  
a polynucleotide encoding a selectable marker;  
a Gram-positive origin of replication; and  
a Gram-negative origin of replication.

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74. A Gram-positive bacteria comprising an expression cassette according to claim 21.

75. A bacteria comprising the vector of claim 49.

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76. A bacteria comprising the vector of claim 73.

77. A method of modifying a Gram-positive organism to produce light, comprising transforming the Gram-positive organism with an expression cassette according to claim 21.

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78. The method of claim 77 further comprising providing the substrate required for *luc*-mediated luciferase activity.

79. A method of screening an analyte for its ability to affect expression of a reporter marker, comprising:  
providing the analyte to Gram-positive bacteria comprising the luciferase expression cassette of claim 21, wherein said reporter marker comprises luciferase;  
providing substrate required for luciferase light production; and  
monitoring the effect of the analyte on the ability of the Gram-positive bacteria to produce light, thereby identifying whether the analyte affects expression of the reporter in Gram-positive bacteria.

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80. The method of claim 79, wherein said substrate is aldehyde and is provided as a vapor.

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81. The method of claim 79, wherein said substrate is a substrate for the *luc* gene product.

82. The method of claim 79, wherein said substrate is (i) aldehyde and is provided as a vapor, and (ii) a substrate for the *luc* gene product.

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83. A method of screening an analyte for its ability to affect expression of a reporter marker in a living, non-human animal, comprising:

introducing Gram-positive bacteria comprising the luciferase expression cassette of claim 21 into the animal, wherein said reporter marker comprises luciferase;

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- 5 providing the analyte to the animal;  
providing substrate required for luciferase light production; and  
monitoring the effect of the analyte on the ability of the Gram-positive bacteria to  
produce light, thereby identifying whether the analyte affects expression of the reporter in  
Gram-positive bacteria in the living, non-human animal.

84. The method of claim 83, wherein said substrate is aldehyde and is provided  
by injection.

- 10 85. The method of claim 83, wherein said substrate is a substrate for the *luc* gene  
product and is provided by injection.

86. The method of claim 83, wherein said substrate is (i) aldehyde and is  
provided as a vapor, and (ii) a substrate for the *luc* gene product.